Fujifilm Dri-Chem Immunoassay Evaluation

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The results of the evaluation of the commercial kit Fujifilm Immuno AG1 for the detection of influenza A virus (Flu A), influenza B virus (Flu B), and human respiratory syncytial virus (HRSV) are summarized below:

- **Study procedure:**

The respiratory samples used in this evaluation were either nasopharyngeal aspirates or nasopharyngeal swabs in a viral transport medium (Universal Viral Transport System, BD™, USA). In agreement with Fujifilm's team, the protocol was adapted to be performed from these liquid specimens (nasopharyngeal aspirate or transport virus medium), and hence, swabs provided in the commercial package were submerged in the specimens, to be subsequently rolled against the wall of the lysis buffer recipient prior to be used in the assay as indicated by the manufacturer.

All samples were analyzed in parallel by Fuji Dri-Chem Immuno AG1 FluA/FluB test (Fujifilm, Japan) as well as for Sofia Influenza A+B FIA (Quidel, USA) test in order to compare these two techniques. Respiratory specimens were additionally tested with a multiplex real-time RT-PCR (Allplex™ Respiratory Panel 1, Seegene, South Korea), which detects 16 different respiratory viruses and determines the Flu A subtypes (H1pdm09, H1 and H3) and HRSV genetic groups (HRSV-A and -B). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. In addition, since Seegene Allplex Assay also provides a Ct (threshold cycle) value, suggestive of the viral load in the specimen, Fujifilm's results could be correlated to the Ct values obtained in the real-time RT-PCR.

Influenza A virus

A total of 102 respiratory specimens were analyzed, of which 35 were PCR-positive for Flu A and 67 were PCR-negative for any respiratory virus. Results are summarized in the following tables:

Table 1: Flu A results by Quidel and Fujifilm's techniques in comparison with Seegene's RT-PCR method.

Flu A		RT-PCR (Seegene)		
		Positive	Negative	Total
FUJIFILM	Positive	17	1	18
	Negative	18	66	84
	Total	35	67	102
QUIDEL	Positive	15	2	17
	Negative	20	65	85
	Total	35	67	102

Table 2: Flu A sensitivity, specificity, PPV and NPV values in comparison with RT-PCR results.

	Sensitivity	Specificity	PPV	NPV
FUJIFILM	48,6%	98,5%	94,4%	78,6%
QUIDEL	42,9%	97,0%	88,2%	76,5%

As shown in Table 2, the specificity, PPV and NPV of both techniques were highly satisfactory as antigen detection tests in comparison to the molecular test. Similar to specificity, PPV and NPV, Fujifilm's sensitivity (48.6%) was higher than Quidel's (42.9%).

Table 3: Correlation between Fujifilm's (A) and Quidel's (B) results and RT-PCR Ct values.

A.

Ct value	22,10	23,27	23,60	23,63	24,99	25,18	26,83	27,22	27,22	27,27
Fujifilm result	+	+	(+)	(+)	+	+	(-)	(+)	(+)	(+)
Ct value	27,32	27,37	27,42	27,77	28,25	28,92	29,11	29,44	29,69	31,37
Fujifilm result	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(-)	(+)
Ct value	31,75	32,19	32,42	32,42	34,72	35,16	35,87	38,90	38,95	
Fujifilm result	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	

B.

Ct value	22,10	23,27	23,60	23,63	24,99	25,18	26,83	27,22	27,22	27,27
Quidel result	+	+	-	+	+	+	-	+	+	+
Ct value	27,32	27,37	27,42	27,77	28,25	28,92	29,11	29,44	29,69	31,37
Quidel result	+	+	+	+	+	-	-	+	-	+
Ct value	31,75	32,19	32,42	32,42	32,96	34,30	34,72	35,16	35,87	36,09
Quidel result	-	-	-	-	-	-	-	-	-	-
Ct value	36,72	37,07	38,90	38,95						
Quidel result	-	-	-	-						

On the other hand, as shown in Table 3, most robust positive Fujifilm's results (in yellow, with a result before the 15-minutes incubation) were mainly detected in those samples with Ct values between 22 and 25. Positive result after whole 15-minutes incubation (in green), were obtained from samples with Ct values between 23 and 31. But, despite most negative results have Ct values above 31, some of them have Ct values from 26 to 29. On the other hand, positive Quidel's results were mainly obtained in those samples with Ct values between 22 and 31, and most negative results have been detected in samples above 29.

Influenza B virus

This part of the evaluation was retrospectively carried out with samples of laboratory sample collection (-80ºC), since influenza B viruses did not circulate during the last season (2016-2017). A total of 102 respiratory specimens were analyzed, 44 were PCR-positive for Flu B, and 58 were PCR-negative for any respiratory virus. Results are summarized in the following tables:

Table 4: Flu B results by Quidel and Fujifilm's techniques in comparison with Seegene's RT-PCR method.

Flu B		RT-PCR (Seegene)		
		Positive	Negative	Total
FUJIFILM	Positive	23	0	23
	Negative	21	58	79
	Total	44	58	102
QUIDEL	Positive	24	3	27
	Negative	20	55	75
	Total	44	58	102

Table 5: Flu B sensitivity, specificity, PPV and NPV values in comparison with RT-PCR results.

	Sensitivity	Specificity	PPV	NPV
FUJIFILM	52,3%	100%	100%	73,4%
QUIDEL	54,5%	94,8%	88,9%	73,3%

As shown in Table 5, Fujifilm's specificity and PPV were maximum, achieving a 100%, which is higher than Quidel's. Fujifilm's NPV were satisfactory, and similar to Quidel's. But, Fujifilm's sensitivity was slightly lower than Quidel's.

Table 6: Correlation between Fujifilm's (A) and Quidel's (B) result and RT-PCR Ct values.

A.

Ct value	22,14	22,43	22,63	23,73	23,92	24,54	25,04	25,04	25,35	25,58	26,41
Fujifilm result	(+)	(+)	+	(+)	+	(-)	(+)	(+)	(+)	(+)	(+)
Ct value	26,58	27,16	27,23	27,85	28,20	28,26	28,52	29,01	29,87	29,94	30,22
Fujifilm result	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(-)	(+)	(+)
Ct value	31,51	31,67	32,06	32,30	32,59	33,64	34,29	36,73	39,06	40,94	
Fujifilm result	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	

B.

Ct value	22,14	22,43	22,63	23,73	23,92	24,54	25,04	25,04	25,35	25,58	26,41
Quidel result	+	+	+	+	+	+	+	+	+	+	+
Ct value	26,58	27,16	27,23	27,85	28,20	28,26	28,52	29,01	29,87	29,94	30,22
Quidel result	+	+	+	+	+	+	+	+	-	+	+
Ct value	31,51	31,67	32,06	32,30	32,59	33,64	34,29	36,73	39,06	40,94	
Quidel result	-	-	+	-	-	-	-	-	-	+	

As shown for Flu A, there is no accurate correlation between the Ct value and the Fujifilm's result but showing a similar trend: the two Fujifilm's robust positive results (in yellow) were mainly detected in samples with a Ct value lower than 23, while most positive results (in green) had Ct values between 22 and 30 in PCR-based assay. Most negative results were associated to high Ct values (31-39), but some negative results were also PCR-positive with Ct values lower than 30. On the other hand, positive Quidel's results were mainly obtained in those samples with Ct values between 22 and 30, and most negative results were detected in samples above 31.

Human respiratory syncytial virus

A total of 104 respiratory specimens were analyzed, of which 70 were positive for HRSV and 34 were negative for any respiratory virus. Results are summarized in the following tables:

Table 7: HRSV results by Quidel and Fujifilm's techniques in comparison with Seegene's RT-PCR method.

HRSV		RT-PCR (Seegene)		
		Positive	Negative	Total
FUJIFILM	Positive	27	1	28
	Negative	43	33	76
	Total	70	34	104
QUIDEL	Positive	30	5	35
	Negative	40	29	69
	Total	70	34	104

Table 8: HRSV sensitivity, specificity, PPV and NPV values in comparison with RT-PCR results.

	Sensitivity	Specificity	PPV	NPV
FUJIFILM	38,6%	97,1%	96,4%	43,4%
QUIDEL	42,9%	85,3%	85,7%	42,0%

Table 9: Correlation between Fujifilm's (A) and Quidel's (B) result and RT-PCR Ct values.

A.

Ct value	19,35	19,48	20,03	20,07	20,30	20,86	20,92	20,97	21,11	21,15	21,98	22,03
Fujifilm result	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Ct value	22,09	22,65	22,71	22,89	22,93	23,14	23,25	23,78	24,19	24,40	24,68	25,60
Fujifilm result	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(+)	(+)	(-)
Ct value	26,03	26,35	27,41	27,64	27,88	28,02	28,48	28,50	28,50	28,78	28,80	28,87
Fujifilm result	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(+)	(+)	(-)
Ct value	29,18	29,97	30,00	30,00	30,10	30,15	30,50	30,50	31,02	32,40	32,60	32,78
Fujifilm result	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Ct value	32,90	33,00	33,20	33,74	34,00	34,50	35,00	35,66	36,00	36,60	36,65	36,73
Fujifilm result	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Ct value	37,12	37,15	37,80	38,10	38,18	39,10	39,38	40,64	41,53	41,98		
Fujifilm result	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)		

B.

Ct value	19,35	19,48	20,03	20,07	20,30	20,86	20,92	20,97	21,11	21,15	21,98	22,03
Quidel result	+	+	+	+	+	+	+	+	+	+	+	+
Ct value	22,09	22,65	22,71	22,89	22,93	23,14	23,25	23,78	24,19	24,40	24,68	25,60
Quidel result	+	+	+	+	+	+	+	-	+	+	+	-
Ct value	26,03	26,35	27,41	27,64	27,88	28,02	28,48	28,50	28,50	28,78	28,80	28,87
Quidel result	+	-	-	-	+	-	-	-	-	+	+	-
Ct value	29,18	29,97	30,00	30,00	30,10	30,15	30,50	30,50	31,02	32,40	32,60	32,78
Quidel result	+	-	-	-	-	-	-	-	+	-	-	-
Ct value	32,90	33,00	33,20	33,74	34,00	34,50	35,00	35,66	36,00	36,60	36,65	36,73
Quidel result	-	-	-	-	-	-	-	-	-	-	-	-
Ct value	37,12	37,15	37,80	38,10	38,18	39,10	39,38	40,64	41,53	41,98		
Quidel result	-	-	-	-	-	+	+	-	-	-		

Fujifilm's specificity and PPV were clearly higher than Quidel's, with similar NPV, but sensitivity was lower (Table 8). Regarding the correlation (Table 9) between positive or negative Fujifilm's results and Ct values, positive results (in green) after whole 15-minutes incubation in the device showed Ct values between 19 and 29, but negative results were detected in samples with Ct values higher than 25. On the other hand, positive Quidel's results were mainly obtained in

those samples with Ct values between 19 and 26, and most negative results were detected in samples above 26.

Comments

In this evaluation two antigen detection tests (Fujifilm AG1 vs. Quidel Sofia) were compared to a molecular test that is able to detect simultaneously Flu A and B viruses, or human respiratory syncytial virus. It is well known that antigen detection tests are less sensitive than molecular techniques, and in this evaluation this feature was also shown. Despite there were not relevant differences, Fujifilm' sensitivity was higher than Quidel's for Flu A (48.6% vs. 42.9%), but not for Flu B (52.3% vs. 54.5%) or HRSV (38.6% vs. 42.9%). This minor sensitivity might be due to the use of a swab for the determination, as previously described, which would be improved using directly the clinical sample. In a 24h emergency room of a central laboratory or of a Microbiology laboratory would be easier to implement from the clinical samples than using a swab. However, all Fujifilm's PPV, NPV and specificity were overall higher than Quidel's, which are valuable features for a point-of-care antigen detection test. In addition, there was not a clear correlation between Ct values and Fujifilm's results, because some Fujifilm's negative samples showed low Ct values, which is suggestive of a high viral load, more in particular for HRSV detection.

In our opinion, Fujifilm AG1 assay is feasible and easy to use, with satisfactory PPV and NPV in comparison with similar point-of-care tests. The fact that the device can provide a rapid result in those samples with high viral load without finishing 15-minutes incubation in the device is one of the most valuable characteristics. Moreover, regarding the hands-on-time required by both techniques, Quidel's protocol requires more steps in handling, since it is needed to prepare the lyophilized reagents prior to be used, thus increasing required manipulation time. In addition, the implementation of this assay in a hospital emergency room or even in primary care centers does not demand a high expertise staff for handling (user-friendly). Perhaps, in the emergency room, the desired feature would be the possibility to test different samples simultaneously by using only one device, with incubation out and a "read now" mode available. This feature is particularly recommended during the peak of HRSV or influenza epidemics, but not required in other period throughout the year. Finally, an aspect to improve of this technique would be the capacity to transmit authomaticaly the results to the laboratory information system (LIS) without the user intervention, or to compile the daily results into a single database on the cloud to be analysed by connectivity solutions similar to Quidel's Virena. This data management would be very useful especially for a hospital with an important workload like ours, or to create a "surveillance network" in case of having devices installed in differents centers in the Primary Care of a city like Barcelona.

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